昆虫学报 *Acta Entomologica Sinica*, November 2012, 55(11): 1282 – 1288 ISSN 0454-6296

Efficacy of entomopathogenic fungi in combination with diatomaceous earth against *Callosobruchus maculatus* (Coleoptera: Bruchidae)

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Abstract: Experiments were conducted to enhance the virulence of two isolates of entomopathogenic fungi, IRAN-187C of *Beauveria bassiana* (Balsamo) Vuillemin, and A-396 isolate of *Metarhizium anisopliae* (Metschnikow) Sorokin, by combination with InsectoSec® formulation of diatomaceous earth (DE) against the adults of *Callosobruchus maculatus* F. under laboratory conditions. The results demonstrated that the combinations between diatomaceous earth and the fungal isolates at lethal and sub-lethal doses achieved high mortality rates in the adult populations; moreover, the treatments improved LT_{50} values of the fungi. The lowest value of LT_{50} (122.2 h) was achieved by LC_{50} (*B. bassiana*, IRAN-187C) + LC_{50} (DE) treatment which was on par with that of LC_{50} (*M. anisopliae*, A-396) + LC_{50} (DE) combination (128.2 h) based on their 95% fiducial limit values, resulting in better efficacy of each fungal isolate in combination with diatomaceous earth against *C. maculatus* adults. Also, effects of the three temperatures (22, 27, and 30℃) and two relative humidity levels (40% and 55%) on the activity of diatomaceous earth when applied against *C. maculatus* adults showed that the least value of LT_{50} (110.11 h) was attained in 30℃ and RH of 40%, suggesting high activity of diatomaceous earth in high temperatures and low humidity conditions.

Key words: *Callosobruchus maculatus*; *Beauveria bassiana*; *Metarhizium anisopliae*; diatomaceous earth; virulence; median lethal concentration (LC_{50}); median lethal time (LT_{50}); mortality

1 INTRODUCTION

Beauveria bassiana (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschnikow) Sorokin are entomopathogens having a broad range of hosts (Lacey *et al.* 2001). These fungi are of low-risk insecticides for human, environment and benefit insects in comparison with common insecticides (Feng *et al.*, 1994). The only factor which loses inoculums resistance of entomopathogenic fungi under field conditions is UV light, that most grain storage does not have this condition (Waseem *et al.*, 2004). The cowpea weevil, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae), is a worldwide pest (Kamel and Pourmirza, 2006), and its larvae develop within various cultivated legumes, such as black eyed beans (*Vigna unguiculata*) (Timms, 1998). Their damage causes loss of weight, nutritional value and viability of stored grains (Swella and Mushobozy, 2007). The controlling of cowpea bruchids in stores has been accomplished by fumigants such as methyl bromide and phosphine. But the use of methyl bromide is being restricted because of its potential to damage the ozone layer. The future use of phosphine also could be threatened by the development of resistant strains of pests (Moravvej and Abbar, 2008). Therefore, insecticide resistance and the consequent losses of food due to failure to control insect pests cause economic losses of several billion dollars worldwide each year (Odeyemi *et al.*, 2006). Accordingly, there is an urgent need to develop safe alternatives to conventional insecticides and fumigants to protect stored products from insect infestations (Moravvej and Abbar, 2008). However, a few researches have been done for the purpose of controlling *C. maculatus* (Lawrence and Khan, 2002). High doses of *B. bassiana* are required for stored grain pests and it may not be attractive for many real-world applications. Thus, there is a need to establish use rates that would be commercially acceptable for the stored grain environment. The efficacy of *B. bassiana* can be increased in different ways. Rice and Cogburn (1999) suggested that the cuticle abrasive materials can increase the efficacy of *B. bassiana*, since *B. bassiana* must adhere to, generate on, and penetrate through the host integument. Waseem *et al.* (2004) hypothesized that the fungal efficacy may be improved in the

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Received: 2012-07-12; Accepted: 2012-11-16

presence of other surface-active agents like diatomaceous earth (DE). In addition, Lord (2001) found that DE has a synergistic interaction with *B. bassiana* against some stored grain pests. In this study, efficacy of Iranian isolates of the fungi, *B. bassiana* (IRAN-187C) and *M. anisopliae* (A-396), has been investigated as alone or in combination with the DE to control *C. maculatus* adults, to assess their impacts under laboratory conditions. Morever, the possible impact of the environmental conditions (temperature and humidity) on the activity of DE has also been evaluated.

2 MATERIALS AND METHODS

2.1 Insects

Adults of *C. maculatus* were obtained from the Department of Plant Protection, Faculty of Agriculture, University of Maragheh. The adults were reared under laboratory conditions in an insectarium (28 ± 2℃, 50% ± 5% RH, 16L: 8D). Adults were used in all the experiments after rearing for five generations.

2.2 Fungal isolates

Isolates of the fungus *B. bassiana* (IRAN-187C) and *M. anisopliae* (A-396) were obtained from the fungal culture collection of Tehran and Urmia universities, Iran. These isolates were originally isolated from soil samples in the municipalities of Sari and Urmia, Iran, respectively. Germination rates were at least 85% throughout the study. The isolates were cultured on Potato Dextrose Agar (PDA) in 9 cm diameter Petri dishes and were used in the bioassays.

2.3 Preparation of conidial suspensions

A mixture of conidia and hyphae with sterile distilled water containing 0.05% (v/v) Tween 80 was prepared in the 50 mL centrifuge tubes. The mixture was vortexed for 5 min to break up the conidial chains or clumps. Conidia were separated from hyphae and substrate materials by filtration of the suspension through three layers of cheese cloths. The concentrations of fungal conidia in suspension were determined using a haemocytometer (Improved Neubauer, 0.1 mm in depth).

2.4 Diatomaceous earth

A sample of InsectoSec® was obtained from Biofa GmbH (Biofa AG is a medium-sized company in south-western Germany). InsectoSec® is a DE formulation of freshwater origin, white and solid powder, insoluble in water, and the dust fineness of less than 100 microns and from chemical point of view contains approximately 90% SiO_2.

2.5 Fungal bioassay

After preliminary assays, five different conidial concentrations were prepared based on the logarithmic series. There were three replications for each treatment. Ten 1-day-old *C. maculatus* adults were inoculated by spraying method with a hand-sprayer and four puffs in each time. As a matter of fact, in the developing countries, warehouse buildings are not well insulated and the stores sanitation is not carefully respected. So, in empty warehouses there are always some food materials which could be enough for insect survival. In such circumstances, spore spraying of the empty warehouses can be considered as a secure method of control before filling them with food materials. Due to this, fungal spore spraying method was implemented in this research. On the other hand, this method was introduced since the dry conidia are not carefully adhering to the surface of insect body. Control insects were also treated using sterile distilled water containing Tween 80 (0.05% v/v). The treated insects were transferred into the 50 mL glass jar containing 15 g cowpea. The glass jars were clogged with sterile cotton swabs and then incubated at 25 ± 1℃ and 70% ± 2% RH. Mortalities were counted for 7 d. Dead insects from each treatment were washed three times in sterile distilled water and kept separately in Petri dishes. The dishes were then incubated in a desiccator with high RH (approximately 100%) to observe the outgrowth of the fungi.

2.6 DE bioassay

For the DE formulation, six concentrations were tested: 10, 20, 40, 80, 100 and 200 mg/kg. DE was added to a glass jar containing 15 g of grains of cowpea (11% – 12% moisture) and then mixed by hand. Ten 1-day-old adults were introduced into each jar. An additional glass jar of untreated cowpea served as untreated controls. Untreated cowpea was used as a control. For every concentration, three replications were performed. Mortalities were counted for 7 d and then dead insects were removed. Experiments were carried out at 25 ± 1℃ and 70% ± 2% RH in the incubator.

2.7 Combination of fungi and DE

After determination of LC_{30}, LC_{50} and LT_{50} values of each fungal isolate and InsectoSec® formulation, combinations were tested between values of LC_{50}(fungi) + LC_{50}(DE), LC_{50}(fungi) + LC_{30}(DE), LC_{30}(fungi) + LC_{50}(DE), and LC_{30}(fungi) + LC_{30}(DE), for evaluation of their behavior and detecting the impact of different amounts of fungi and DE on the level of median lethal time. For example, for combination of LC_{50} (fungi) + LC_{50} (DE), adults were exposed to the LC_{50} concentration value of the fungus, then 15 g of cowpeas were mixed with the LC_{50} concentration value of DE and ten 1-day-old adults were placed into the grain in each 50 mL glass jar. Control insects were treated using sterile distilled water containing Tween 80 (0.05%, v/v). Each treatment and untreated controls had three replications per experiment and each experiment was replicated at least three times with different generations of insects. The experiments were placed in an incubator with conditions of 25 ± 1℃ and 70% ± 2% RH. Mortality was counted for 7 d and dead insects were removed and placed in a desiccator with humid saturated condition to observe the outgrowth of fungi as per described above. For other combinations similar manner was performed as explained earlier.

2.8 Effects of temperature and humidity on the activity of DE

Exposure studies were conducted at three temperatures, *viz.*, 22, 27, and 30℃ and two relative humidity levels, *viz.*, 40 and 55 percent as six temperature-humidity combinations. For each trial with a particular temperature-humidity combination, 15 g of cowpea were placed in each of 50 mL glass jar, and the fixed amount of InsectoSec® (equal with LC_{50}) was added to the cowpea. Ten 1-day-old adults were introduced into each jar. Treatments were placed in the incubators with 3 temperatures and 2 humidity levels. The beetles were exposed for 7 d and mortalities were counted. An untreated control was also conducted for each treatment. Each treatment replicated thrice.

2.9 Data analyses

Cumulative mortality counts obtained from the experiments were corrected by using Abbott's formula (Abbott, 1925). Data were statistically analyzed using ANOVA (SAS Institute, 2000) and means were separated using the Duncan's multiple range test at $P = 0.05$. Probit analyses were used to estimate LC_{30}, LC_{50} and LC_{90} values of the isolates and DE with 95% confidence limits (CL) as well as LT_{50} values (SPSS 17).

3 RESULTS

3.1 Effect of fungal isolates on *C. maculatus* adults

Bioassays of the isolates of fungi against the adults of *C. maculatus* by spraying method under laboratory condition revealed different variations in their biological activity, in which the mortality rates enhanced with increasing conidial concentrations. The IRAN-187C isolate of *B. bassiana* was the most virulent with the lowest LC_{50} (4.24×10^7 conidia/mL) and LT_{50} values (Tables 1, 2 and 3). The LC_{50} value of *M. anisopliae* A-396 was calculated as 9.60×10^7 conidia/mL (95% CL = 5.83×10^7 – 1.46×10^8). The LT_{50} value recorded for *B. bassiana* IRAN-187C at 4×10^8 conidia/mL concentration was 132 h, but it was calculated 134 h for *M. anisopliae* A-396 at the concentration of 5×10^9 conidia/mL (Table 3).

3.2 Effect of DE on *C. maculatus* adults

Mortality rates were increased as the concentration of DE enhanced (Table 1). The median lethal concentration of DE was calculated 38 mg/kg, showing higher sensitivity of *C. maculatus* to the InsectoSec® formulation. The LT_{50} value of DE alone was detected 139 h for cowpea weevils (Table 3).

3.3 Effect of combination of fungi and DE on *C. maculatus* adults

All the combinations of *B. bassiana* IRAN-187C and DE, *viz.*, LC_{50} (fungi) + LC_{50} (DE), LC_{50} (fungi) + LC_{30} (DE), and LC_{30} (fungi) + LC_{50} (DE), on the *C. maculatus* adults showed a mortality rate of 69 to 100 percent; whereas, mortalities in high concentrations of *B. bassiana* (4×10^8 conidia/mL) and DE (200 mg/kg) were recorded 84.4% and 85%, respectively (Table 3). When 4.24×10^7 conidia/mL (LC_{50}) of *B. bassiana* IRAN-187C was combined with a DE concentration of 38 mg/kg (LC_{50}), mortality increased to 100% (Table 3). In addition, the least median lethal time (122.2 h, fiducial limits: 112 – 133.3 h) was achieved by the combination of LC_{50} (fungi) + LC_{50} (DE), it is significantly comparable with those of the fungus and DE as alone, 134 h and 139 h, respectively (Table 3). These results demonstrate an incremental effect of DE on the fungi. Median lethal time was 126 h when the LC_{30} values (17 mg/kg) of DE were combined with LC_{50} value of *B. bassiana*. In the combination, mortality was achieved at 83.5%. However, the highest median lethal time (158 h) and cumulative mortality rate (69.2%) were achieved by LC_{30} (*B. bassiana*) + LC_{30} (DE) combination (Table 3). Similar results were attained from the combination of *M. anisopliae* A-396 and DE. Mortality at the highest dose of *M. anisopliae* (5×10^9 conidia/mL) was calculated as 91.63%. While combination of LC_{50} values of *M. anisopliae* (9.60×10^7 conidia/mL) and DE (38 mg/kg) resulted in 100% mortality and the LT_{50} value decreased to 128.2 h (Table 3).

Table 1 Cumulative percentage mortalities of *Callosobruchus maculatus* adults 7 d after treatments with *Beauveria bassiana*, *Metarhizium anisopliae* (conidia/mL) and InsectoSec® formulation of DE (mg/kg)

Treatments		Mortality (%)
B. bassiana IRAN-187C (conidia/mL)	1.0×10^{7}	22.9 ± 8.0 d
	2.5×10^{7}	46.0 ± 3.3 c
	6.3×10^{7}	57.4 ± 12.0 c
	1.6×10^{8}	76.8 ± 5.7 b
	4.0×10^{8}	84.4 ± 3.3 a
M. anisopliae A-396 (conidia/mL)	1.0×10^{7}	33.2 ± 12.0 c
	6.6×10^{7}	41.6 ± 6.7 bc
	4.2×10^{8}	66.7 ± 12.0 b
	2.6×10^{9}	87.5 ± 5.7 a
	5.0×10^{9}	91.6 ± 3.3 a
InsectoSec® (mg/kg)	10	23.0 ± 6.7 d
	20	34.7 ± 12.0 c
	40	46.0 ± 14.5 c
	80	53.6 ± 0.0 c
	100	76.6 ± 11.5 b
	200	85.0 ± 6.7 a

Means followed by different letters in a row are significantly different ($P = 0.05$) by DMRT.

Table 2 Probit analysis of concentration-mortality response of *Callosobruchus maculatus* adults to *Beauveria bassiana* and *Metarhizium anisopliae* isolates and InsectoSec® formulation of DE

Treatments	LC$_{50}$	95% Fiducial limits		Slope ± *SE*	LC$_{30}$	LC$_{90}$
		Lower	Upper			
B. bassiana IRAN-187C (conidia/mL)	4.24×10^{7}	3.29×10^{7}	5.32×10^{7}	1.28 ± 0.10	1.66×10^{7}	4.22×10^{8}
M. anisopliae A-396 (conidia/mL)	9.60×10^{7}	5.83×10^{7}	1.46×10^{8}	0.70 ± 0.07	1.82×10^{7}	5.54×10^{9}
InsectoSec® (mg/kg)	38	8.91	85.66	1.49 ± 0.40	16.85	273

Table 3 Probit analysis of time-mortality response of *Callosobruchus maculatus* adults and related cumulative percentage mortalities to the fungal isolates and InsectoSec® formulation of DE and their combinations

Treatments		LT$_{50}$ (h)	95% Fiducial limits		CM% ± *SE*
			Lower (h)	Upper (h)	
B. bassiana (IRAN-187C)[*]		134.0	117.4	154.4	84.4 ± 3.3
M. anisopliae (A-396)[#]		132.0	118.5	147.3	91.6 ± 3.3
InsectoSec®		139.0	132.6	148.6	85.0 ± 6.7
B. bassiana + DE	LC$_{50\ (\mathrm{Fungi})}$ + LC$_{50\ (\mathrm{DE})}$	122.2	112.0	133.3	100.0 ± 0.0
	LC$_{50\ (\mathrm{Fungi})}$ + LC$_{30\ (\mathrm{DE})}$	126.0	111.6	141.5	83.7 ± 8.8
	LC$_{30\ (\mathrm{Fungi})}$ + LC$_{50\ (\mathrm{DE})}$	139.4	116.0	149.0	79.7 ± 5.7
	LC$_{30\ (\mathrm{Fungi})}$ + LC$_{30\ (\mathrm{DE})}$	158.0	153.6	163.5	69.2 ± 3.3
M. anisopliae + DE	LC$_{50\ (\mathrm{Fungi})}$ + LC$_{50\ (\mathrm{DE})}$	128.2	106.7	156.8	100.0 ± 0.0
	LC$_{50\ (\mathrm{Fungi})}$ + LC$_{30\ (\mathrm{DE})}$	130.3	120.8	140.8	95.8 ± 3.3
	LC$_{30\ (\mathrm{Fungi})}$ + LC$_{50\ (\mathrm{DE})}$	131.4	126.8	136.2	92.0 ± 3.3
	LC$_{30\ (\mathrm{Fungi})}$ + LC$_{30\ (\mathrm{DE})}$	153.0	147.8	161.0	68.0 ± 5.7

[*] All the treatments contained *Beauveria bassiana* at 4×10^{8} conidia/mL; [#] All the treatments contained *Metarhizium anisopliae* at 5×10^{9} conidia/mL; ® All the treatments contained InsectoSec® at 200 mg/kg.

3.4 Effect of temperature and humidity on the activity of DE against *C. maculates* adults

An experiment was conducted to evaluate the effects of three temperature and two humidity levels on the activity of DE. At 22℃ DE (38 mg/kg) caused 100 percent mortality of *C. maculates* adults at 40% RH but at 55% RH the mortality (69%) was significantly lower. Another two temperatures (27 and 30℃) showed different effects representing no significant difference between the two RH levels (Table 4).

Comparison of LT_{50} values revealed that the least and highest median lethal time (110.1 h and 148.7 h) occurred at 30℃ and 40% RH, and 22℃ and 55% RH, respectively, causing 100% and 69% mortalities on the adults (Table 4). Analyses of variance for these parameters were performed as given in Table 5.

Table 4 Probit analysis of time-mortality response of *Callosobruchus maculatus* adults and related cumulative percentage mortalities to the InsectoSec® formulation of DE (at its LC_{50} value: 38 mg/kg) at three different temperatures and two humidity rates

Temperature (℃)	Relative humidity (%)	LT_{50} (h)	95% Fiducial limits		CM%± *SE*
			Lower (h)	Upper (h)	
22	40	115.2	98.0	131.5	100.0 ±0.0 a
	55	148.7	144.3	153.6	69.0 ±3.3 b
27	40	113.8	71.8	147.6	91.8 ±3.3 ab
	55	138.0	130.9	145.6	95.8 ±3.3 ab
30	40	110.1	61.9	165.5	100.0 ±0.0 a
	55	114.5	74.9	169.4	100.0 ±0.0 a

Table 5 Analyses of variance (ANOVA[b]) for effect of temperature and humidity on the activity of DE

Model		Sum of squares	*Df*	Mean square	*F*	Sig.
1	Regression	2 552.550	2	1 276.275	5.910	0.091[a]
	Residual	647.856	3	215.952		
	Total	3 200.406	5			

[a] Predictors: (Constant), humidity, temperature; [b] Dependent variable: LT_{50}.

4 DISCUSSION

Bioassays against the adults of *C. maculatus* with two fungal isolates of *B. bassiana* and *M. anisopliae* revealed the existence of different levels of virulence as reflected in their biological activities showing susceptibility of the species to the tested fungal isolates. Vilas Boas *et al.* (1996) tested *B. bassiana* and *M. anisopliae* isolates against *C. maculatus* adults. In the experiment *B. bassiana* PL256 had a shorter LT_{50} value than *M. anisopliae* PL43. In the other test by Cherry *et al.* (2005), 8 isolates of *B. bassiana* and 4 isolates of *M. anisopliae* were used against *C. maculatus* adults. The study showed *B. bassiana* to be the more virulent agent against *C. maculatus* than *M. anisopliae*. Our results are in consistence with those of the previous studies, in which these fungi have different levels of virulence indicating that *B. bassiana* can be more successful than *M. anisopliae* for infection of cowpea weevils. Our research also showed high lethal efficacy of InsectoSec® formulation of DE on the *C. maculatus* adults. As a result, susceptibility of a given species to DE is influenced by formulation of DE, exposure interval, environmental conditions (temperature and humidity) and the characteristics of its cuticle. Comparison of susceptibility of *Tribolium castaneum* and *Sitophilus oryzae* by Fields and Korunic (2000) proved that *T. castaneum* was more tolerant than *S. oryzae*. Other researchers also reported that sensitivity of different species of insects to DE depends on their cuticle lipid structure (Mewis and Ulrichs, 2001). Arthur (2001) evaluated the effects of three temperatures (22, 27 and 32℃) and three relative humidity levels (40%, 57% and 75%) on susceptibility of *Oryzaephilus surinamensis* (L.) adults exposed to DE (Protect-ItTM), and reported that mortality of *O. surinamensis* directly increased as temperature amplified. Insect activity usually increases as temperature increases; therefore, insects can coat with more quantity of the diatomaceous earth as they became more active.

Furthermore, this study showed that the mortality was lower at 75% RH compared to that at 40% and 57% RH. Reviews of studies in which stored-product beetles have been exposed to diatomaceous earths reported a general decrease in efficacy with an increase in grain moisture content or relative humidity (Arthur, 2001). Athanassiou (2005) reported that in addition to impact of temperature and humidity on the activities of DE, exposure interval is also important and mortality could get increase as exposure interval enhances.

Results from these studies are on a par with those of others in which high mortality is achieved by increasing and decreasing in temperature and relative humidity, respectively. To evaluate the efficacy of three temperatures (22, 27 and 30℃) and two relative humidity rates (40% and 55%) on DE activity against *C. maculatus* adults, the least amount of LT_{50} value was achieved in 30℃ and 40% RH which caused 100 percent mortality. These results show high activity of DE in high temperatures and low humidity. Among the impacts of temperature and relative humidity, the effect of temperature seems to be as effective as humidity between the two ranges of tested humidity levels, because the increase in the relative humidity from 40% to 55% at the least tested temperature (22℃) caused significant decrease in the mortalities. However, at other temperatures evaluated in the study, increase in humidity level showed no significant decrease in the adults' mortality.

The results of combination of the fungi with DE showed that DE could effectively potentiate the fungal activity. These are similar to the results of the other studies. Waseem *et al.* (2004) combined a fixed dose of diatomaceous earth (190 mg/kg) with different *B. bassiana* concentrations ranging from 33 to 2 700 mg of conidia per kg of grain against the *T. castaneum* larvae and adults at two different relative humidity rates, *viz.*, 56% and 75% RH. Both adults and larvae of *T. castaneum* proved to be very tolerant to *B. bassiana* and DE when they were tested alone; whereas, the presence of DE resulted in 17 and 16-fold reduction in the median lethal concentration of *B. bassiana* at 56% and 75% RH, respectively. They identified two factors that contribute to the synergism. Spore attachment to larvae is significantly greater in the presence of diatomaceous earth than without it, and the lipids that were taken up by diatomaceous earth included phospholipids, confirming that damage to the insect cuticle makes subcuticular nutrients available to conidia for their germination. Similarly, Lord (2001) has reported the presence of a synergistic interaction in combination of 200 mg/kg of DE with *B. bassiana* at rates from 11 to 300 mg conidia/kg against *O. surinamensis* and *Rhizopertha dominica*. These results will help us to develop a strategy for using a combination of environmentally benign materials such as entomopathogenic fungi and diatomaceous earth to control insect pests of stored products.

ACKNOWLEDGMENTS We are grateful to Dr. G. Gharekhani for providing the diatomaceous earth formulation for the experimentations.

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昆虫病原真菌与硅藻土混用对四纹豆象的毒力效果

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摘要: 在室内条件下检测了白僵菌 *Beauveria bassiana* (Balsamo) Vuillemin 菌株 IRAN-187C 和金龟子绿僵菌 *Metarhizium anisopliae* (Metschnikow) Sorokin 菌株 A-396 分别与硅藻土(DE)制剂 InsectoSec® 混用对四纹豆象 *Callosobruchus maculatus* 成虫的毒力效果。结果表明:硅藻土与致死剂量和亚致死剂量的真菌分离物混用导致了四纹豆象成虫的高死亡率,且这些处理均缩短了真菌的致死中时(LT_{50})。LC_{50}的白僵菌 *B. bassiana* 菌株 IRAN-187C 和 LC_{50}的 DE 混用对四纹豆象的 LT_{50}(122.2 h)最低,在 95% 置信限上与 LC_{50}的金龟子绿僵菌 *M. anisopliae* 菌株 A-396 与 LC_{50}的 DE 混用得到的 LT_{50}(128.2 h) 相当,说明两种真菌分离物与 DE 混用对四纹豆象成虫都具有较好的毒力效果。用四纹豆象成虫检测 3 个温度(22, 27 和 30℃)和 2 个相对湿度(40% 和 55%)组合对 DE 活性的影响,结果显示 30℃ 和 40% 相对湿度条件下得到的 LT_{50}(110.11 h)最低,说明高温低湿条件下 DE 活性较高。

关键词: 四纹豆象; 白僵菌; 金龟子绿僵菌; 硅藻土; 毒力; 致死中浓度; 致死中时; 死亡率

中图分类号: Q965.9 **文献标志码**: A **文章编号**: 0454-6296(2012)11-1282-07

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